Phase Contrast Microscopy Coupled to Image Analysis as a Rapid Method to Monitor Wine Flora

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In food industry, there is a need for the development of rapid methods to monitor microflora at any time during processing. Conventional methods are time consuming and have poor sensitivity and specificity. The use of routine microscopic techniques coupled to image analysis is a powerful method that has already been applied successfully to several industries.

Vinho Verde wines, together with Port wines, provide Portugal's main wine exports. *Vinho Verde* wines are characterised by having a relatively low alcohol content and quite a high malic acid content. High levels of acidity are sometimes undesirable. To control the acidity of the wines, deacidification process can be done using lactic acid bacteria that convert malic acid to lactic acid – the malolactic fermentation.
The presented results describe the use of phase contrast microscopy coupled to image analysis to simultaneously monitor bacteria and yeast during *Vinho Verde* fermentation.

Several factors had to be considered during software development – different size and motility of bacteria and yeast as well as different brightness and the presence of impurities. To solve this problem an algorithm for each of the species under consideration was prepared. For the counting of bacteria, a method based on the difference between two consecutive images caused by bacteria movement was developed. For yeasts, a two-stage process was prepared. Firstly, all unwanted pixels were eliminated and then individual objects were individualised and counted.

This method has proved to be suitable for simultaneous counting of lactic acid bacteria and yeasts.

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